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International Journal of Phytoremediation

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713610150>

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First published on: 01 July 2005

To cite this Article Zhang, Lan , Angle, J. Scott , Delorme, Thierry and Chaney, Rufus L. (2005) 'Technical Note: Degradation of *Alyssum Murale* Biomass in Soil', International Journal of Phytoremediation, 7: 3, 169 — 176, First published on: 01 July 2005 (iFirst)

To link to this Article: DOI: 10.1080/16226510500214475

URL: <http://dx.doi.org/10.1080/16226510500214475>

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Technical Note

DEGRADATION OF *ALYSSUM MURALE* BIOMASS IN SOIL

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The Ni-hyperaccumulating plant Alyssum murale accumulates exceptionally high concentrations of nickel in its aboveground biomass. The reasons for hyperaccumulation remain unproven; however, it has been proposed that elemental allelopathy might be important. High-Ni leaves shed by the plant may create a “toxic zone” around the plant where germination or growth of competing plants is inhibited. The efficacy of this argument will partially depend upon the rate at which leaves degrade in soil and free metals are released, and the subsequent rate at which metals are bound to soil constituents. To test the degradation of biomass of hyperaccumulators, A. murale was grown on both high- and low-Ni soils to achieve high- (12.0 g Ni/kg) and low- (0.445 g Ni/kg) Ni biomass. Shredded leaf and stem biomass were added to a serpentine soil from Oregon that was originally used to grow high-Ni biomass and a low-Ni control soil from Maryland. Biomass Ni was readily soluble and extractable, suggesting near immediate release as biomass was added to soil. Extractable nickel in soil amended with biomass declined rapidly over time due to Ni binding in soil. These results suggest that Ni released from biomass of Ni hyperaccumulators may significantly affect their immediate niche only for short periods of time soon after leaf fall, but repeated application may create high Ni levels under and around hyperaccumulators.

KEY WORDS: hyperaccumulator biomass, extractable nickel, elemental allelopathy, *Alyssum murale*

INTRODUCTION

Hyperaccumulators accumulate metals to exceptional levels, greatly exceeding those normally considered to be phytotoxic in aboveground tissues (Baker and Brooks, 1989; Brooks *et al.*, 1977). Reeves (1992) suggested as a widely accepted definition of Ni hyperaccumulators “a plant in which a nickel concentration of at least 1000 mg/kg has been recorded in the dry matter of any above-ground tissue in at least one specimen growing in its natural habitat.” *Alyssum murale* has received wide attention because of its extraordinary ability to extract and accumulate Ni from contaminated soils (Chaney *et al.*, 1999; Morrison *et al.*, 1980).

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Uptake of metals from soils by hyperaccumulators does not appear to be a passive phenomenon. Explanations for the phenomenon of metal hyperaccumulation have been suggested, including inadvertent uptake, metal tolerance, disposal from the plant body, drought resistance, pathogen/herbivore defense, and interference between neighboring plants (Boyd and Martens, 1992). Except for an evaluation of the defense hypothesis, other hypotheses are relatively unexplored (Boyd and Jaffré, 2001).

Interference is a biotic interaction that may be mediated by metal hyperaccumulation. Baker and Brooks (1989) suggested that interference may occur between hyperaccumulators and neighboring plants, but they did not offer a mechanism. Wilson and Agnew (1992) suggested that elevated soil metal levels beneath hyperaccumulator canopies exclude less metal-tolerant plants and provide a competitive advantage for the hyperaccumulator. Similarly, Boyd and Jaffré (2001) reported higher Ni levels in surface soil under the canopy of the New Caledonia Ni hyperaccumulator *Sebertia acuminata*, compared to soil taken from under the canopy of nonhyperaccumulator species. Boyd and Martens (1998) suggested that this mechanism is equivalent to “elemental allelopathy.” Compared to classical allelopathy, inhibition in the case of metal hyperaccumulators would be due to redistribution of an element in the soil, rather than manufacture of an organic compound.

The localization and speciation of nickel in plant tissue of hyperaccumulators have been widely investigated. With regard to nickel hyperaccumulators, Ni is preferentially distributed in the epidermal cells, most likely in the vacuoles of the leaves and stems where Ni cannot damage sensitive cellular organelles (Baker *et al.*, 1988; Krämer *et al.*, 2000; Küpper *et al.*, 2001). Nickel is rendered inactive by complexing with amino acids and carboxylic acids, such as histidine, citrate, malate, and oxalic acids (Homer *et al.*, 1991; Jaffré *et al.*, 1976; Krämer *et al.*, 1996).

Although it is likely that some cellular Ni is remobilized out of leaf tissue prior to senescence, leaves shed by hyperaccumulators contain elevated levels of Ni, with resulting high Ni in the soil around the plant. However, the concentration of Ni absorbed by plants is more directly related to the concentration of soluble ions of Ni and the rate of replenishment of this mobile pool (Duneman *et al.*, 1991). The objective of the current study was to mimic conditions under which Ni biomass might be added to soil and to assess the fate of biomass-bound Ni in *A. murale* by determining phytoavailable Ni concentration following biomass incorporation into soil.

MATERIALS AND METHODS

Soil Sampling and Preparation

Two soils were used in this study:

1. Oregon Soil (Brockman variant very gravelly loam; fine, magnesian, mesic Typic Xerochrepts), a Ni-rich serpentine soil collected near Cave Junction, Oregon;
2. Maryland Soil (Christiana fine sandy loam; clayey, kaolinitic, mesic Typic Pseudults), a low-Ni soil collected at Beltsville, Maryland.

Soils were mixed in large containers and dried at room temperature. Rocks were removed from soils and then soils were crushed to pass a 4-mm sieve.

For analyses of soil properties, dry soil samples were ground and passed through a 2-mm sieve. Five g air-dried soil was digested with 10 ml concentrated HNO₃ and heated to near dryness on a hotplate, subsequently dissolved in 20 ml 3 M HCl and heated at

mild reflux for 2 h. The residue was filtered and diluted to 50 ml with 0.1 M HCl. Total nickel concentrations were determined using flame atomic absorption spectrometry (AAS) [Association of Official Analytical Chemists (AOAC) method 3.014 (a), 1984]. Other chemical characteristics of soils were determined by the Soil Testing Laboratory, University of Maryland College Park, MD. Soil pH was measured in a soil water suspension of 1:1 water to soil (Eckert and Sims, 1995). Organic matter content was determined by loss on ignition (Storer, 1984). Mg, P, K, and Ca were extracted with Mehlich (I) and determined with a Technicon Auto-Analyze. Mg and P were measured by a colorimeter, while K and Ca were measured by flame photometry (Flanney and Markus, 1980; Mehlich, 1953). Nitrate nitrogen was measured using a cadmium reduction column in a Technicon Auto-Analyzer System (Keeney and Nelson, 1982).

Collection and Preparation of Plant Biomass

Alyssum murale biomass (high Ni biomass) was collected at Cave Junction, Oregon, from two-year-old plants grown on the high-Ni soil. Shoots were cut 10 cm from the soil surface and placed into paper bags for drying. Biomass was dried for 48 h at 60°C, then ground to pass a 1-mm sieve. The same species was grown on low-Ni soil at Beltsville, MD and the harvest procedure was similar. Although grinding and sieving of biomass does not resemble natural leaf fall and decomposition, it was necessary for homogeneity when mixing soils and biomass.

Carbon, hydrogen, and nitrogen content of biomass were analyzed by the combustion method at the Soil Testing Laboratory, University of Maryland College Park, MD (Campbell, 1992). The other elemental analyses were performed following AOAC method 3.014 (a) (1984). Two grams of biomass were ashed in a muffle oven at 480°C for 16 h. The ash was digested with 2 ml concentrated HNO₃ and heated to near dryness on the hotplate, subsequently dissolved in 10 ml 3 M HCl and refluxed for 2 h on the hotplate. The residue was filtered and diluted to 25 ml with 0.1 M HCl. Elemental concentrations were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) using cobalt as an internal standard.

Extractable Nickel Concentrations in Soil

Each sample (low Ni biomass or high Ni biomass) and each of two soils (Oregon soil or Maryland soil) were added to cups, where 0.475 g biomass and 20 g soil achieved a rate of 0.2 kg dry weight biomass/m² soil. This rate is equal to approximately 20 t fresh weight biomass/ha soil and is similar to yields we have observed in the field. The biomass and soil were mixed to homogeneity. Two soils without biomass addition were also tested as a control. All cups were placed into a growth chamber for incubation. The growth chamber was set at 24°C and 60% relative humidity, with 14 h photoperiod. The temperature and relative humidity remained constant. The moisture of the mixture of soil and biomass was adjusted to 60% water-holding capacity of soil. The water-holding capacities of Oregon and Maryland soils were determined to be 24.4% and 43.7%, respectively (Forster, 1995).

For determining Ni degradation of biomass in soil over time, on days 0, 1, 3, 7, 12, 20, and 30 following biomass addition to soils, cups that included the six treatments were removed from the chamber and air dried at room temperature overnight. For each treatment, Ni was extracted using distilled water and 0.01 M strontium nitrate. Each treatment extracted by one method was replicated four times. Strontium nitrate extractable Ni has been shown

Table 1 Physical and chemical characteristics of soils used to assess metal release from biomass. Means within the column followed by different letters are significantly different at $P < 0.05$ using the student's t -test ($n = 4$)

Soil	Organic matter %	pH	mg/kg					
			NO ₃ -N	Mg	P	K	Ca	Ni
Oregon	4.0	6.5	11.1	81.9	3.40	31.8	489	2.92×10^3
Maryland	2.8	6.0	35.3	161	99.2	161	624	5.54

to be well correlated with Ni concentrations in plant shoots (such as oat, wheat, etc.) and it generally represents phytoavailable Ni in soil (Kukier and Chaney, 2001). The water extract was used as a comparison. Twenty-gram air-dried samples were mixed with 40 ml of extraction solution at a soil:solution ratio of 1:2 and shaken for 1 h, then slurries were filtered using Ahlstrom #513 fluted filter paper inside a #40 Whatman filter paper. The extracted solutions were refrigerated at 4°C until analyzed. Extractable Ni concentrations were determined using AAS. High Ni biomass and low Ni biomass without soils also were extracted with both distilled water and 0.01M strontium nitrate. Chemical properties of soils are shown in Table 1.

Statistical Analyses

Data were analyzed using SAS version 8.01 (SAS Institute, Cary, NC). All values reported in the following tables and figures are means based on four replicates. The differences between means were tested using the student's t -test. Strontium nitrate extractable Ni and soluble Ni from soil amended with biomass over time were compared with analysis of variance (ANOVA) using the Mixed procedure. The effect of time on extractable Ni from soil amended with biomass was tested using the one-way ANOVA F -test. A significance level of $P < 0.05$ was selected for determination of statistical differences.

RESULTS AND DISCUSSION

Alyssum murale was grown on both high- and low-Ni soils to achieve high and low Ni biomass. The total Ni concentrations of high Ni biomass and low Ni biomass were 12.0 g Ni/kg dry biomass and 0.445 g Ni/kg dry biomass, respectively (Table 2). The low Ni biomass still contained moderately high concentrations of Ni. This demonstrates the unique ability of this species to scavenge Ni from low-Ni soil. The results using strontium nitrate and distilled water to extract Ni directly from low Ni or high Ni biomass (without soil) showed that there was no significant difference between water soluble and Sr(NO₃)₂ extractable Ni from biomass. (For low Ni biomass, $P = 0.51 > 0.05$; for high Ni biomass,

Table 2 Macro and micro elemental content or concentration of *A. murale* grown on high- and low-Ni soils. Means are significantly different at $P < 0.05$ using the student's t -test ($n = 4$). All pairs of element contents of high Ni biomass and low Ni biomass are significantly different, except Cu

Biomass	%			g/kg				mg/kg				
	C	H	N	Mg	P	Ca	Ni	Fe	Mn	Cu	Co	Zn
High Nickel	40.7	5.35	4.53	2.23	1.11	20.0	12.0	622	31.4	1.96	7.89	47.7
Low Nickel	45.6	5.86	3.56	1.02	1.92	22.8	0.445	82.3	45.1	2.36	1.86	30.3

Table 3 Extractable nickel (means \pm standard error, $n = 4$) from biomass of *Alyssum murale*. Means within the column followed by the same letter are not significantly different at $P > 0.05$ using the student's t -test

	High Ni biomass		Low Ni biomass	
	g/kg	% (Total Ni)	g/kg	% (Total Ni)
Water soluble Ni	8.62 \pm 0.37	72.1	0.445 \pm 0.017	100
Sr(NO ₃) ₂ extractable Ni	8.71 \pm 0.31	72.8	0.441 \pm 0.040	99.1

$P = 0.73 > 0.05$.) Greater than 70% Ni was extractable in the high Ni biomass, while for the low Ni biomass, nearly all Ni was extractable (Table 3). This suggests that Ni from biomass is highly soluble; thus, when biomass is added to soil, Ni is quickly made phytoavailable. The soluble Ni is potentially phytotoxic, yet it can also quickly interact with other components of the soil.

Although total Ni in the Oregon soil was very high (2.92 g/kg dry soil, Table 1), water-soluble Ni was less than 0.15 mg/kg dry soil and Sr(NO₃)₂ extractable Ni was approximately 2.0 mg/kg dry soil (Figure 1). For Maryland soil, total Ni was 5.54 mg/kg dry soil and both Sr(NO₃)₂ extractable Ni and water-soluble Ni were less than 0.13 mg/kg dry soil. This observation demonstrated the high capacity of the Oregon soil to bind Ni, since only about 0.07% of the total Ni was in the phytoavailable form. The amounts of extractable Ni from both of soils were very small and showed no significant change over time (Figure 1).

For the treatment where the high Ni biomass was added to the Maryland soil, at day 0, Sr(NO₃)₂ extractable Ni was 82.0 mg/g dry soil. Sr(NO₃)₂ extractable Ni in the Oregon soil amended with high Ni biomass was 47.8 mg/kg dry soil (Figure 1). While Sr(NO₃)₂ extractable Ni directly from high Ni biomass was 8.71 g/kg dry biomass (Table 3), the addition of 0.475 g high biomass was expected to increase extractable Ni in soil by about 200 mg/kg soil if there was no interaction between soil and biomass. This indicates that Ni from biomass was immediately bound to soil. Rapid binding further demonstrates that the Oregon soil has strong ability to bind Ni, thus explaining the low extractable Ni concentration in the Oregon soil despite its high total Ni content. Nickel is immediately bound to soil colloids, organic matter, or Fe/Mn oxides, which have shown strong ability to bind Ni in soil (Echevarria *et al.*, 1998; Weng *et al.*, 2001).

At day 0, following low Ni biomass addition to soils, biomass was expected to contribute about 10 mg extractable Ni/kg dry soil if there was no interaction between soil and biomass. In fact, Sr(NO₃)₂ extractable Ni in Maryland and Oregon soils amended with low Ni biomass were 3.26 and 5.74 mg Ni/kg dry soil, respectively (Figure 1). Therefore, data from both the Oregon and Maryland soils amended with the high and low Ni biomass demonstrate that once Ni biomass was mixed with soils, extractable Ni decreased very rapidly, for reasons noted above. For each combination of soil and biomass, water-soluble Ni concentrations were correlated to Sr(NO₃)₂ extractable Ni concentrations over time and there was no significant difference between extraction with water and strontium nitrate (all $P > 0.1$, data not shown).

Strontium nitrate extractable nickel from all amended soils rapidly decreased over time, with few changes beyond 12 d (Figure 1, all $P < 0.001$). For both soils, equilibrium concentrations of Sr(NO₃)₂ extractable Ni in soil amended with high Ni biomass were around 2.5 mg Ni/kg dry soil, while for the low Ni biomass amended soil, equilibrium Sr(NO₃)₂ extractable Ni concentrations were very close to extractable Ni without the addition of biomass, sometimes even lower than the control soil.

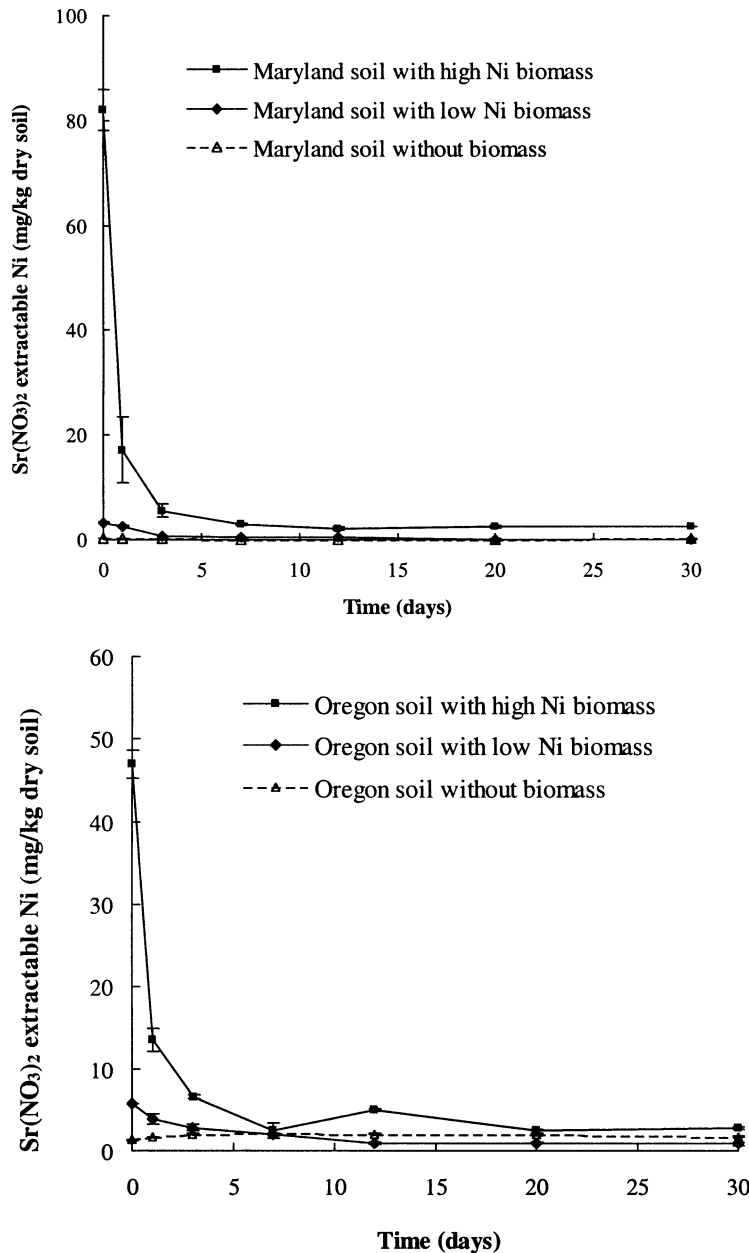


Figure 1 Sr(NO₃)₂ extractable Ni in Maryland and Oregon soils amended with high and low Ni biomass. Values are means ($n = 4$) with vertical bars showing ± 1 s.e.

Although the concentration of extractable Ni in soil was not highly elevated following a single addition of biomass to soil, repeated years of addition as would normally occur with perennial plants such as *Alyssum* could potentially create high Ni levels under and around a hyperaccumulator plant or patch. Indeed, this has previously been shown by others. High soil Ni levels have been reported under the canopy of nickel-hyperaccumulating

plants compared to nonhyperaccumulator species (Boyd and Jaffré, 2001; Krämer *et al.*, 1997; Schlegel *et al.*, 1992). The higher phytoavailable Ni level may cause more Ni to be absorbed by less metal-tolerant plants, resulting in Ni toxicity. Nickel toxicity has been shown to inhibit root growth, depress shoot and leaf growth, and cause general chlorosis of younger leaves (Baker and Walker, 1989). It is therefore possible that elemental allelopathy as suggested by Boyd and Martens (1998) may be another ecologic advantage conferred by the process of metal hyperaccumulation. The current study shows that Ni is rapidly released from leaf tissue. The Ni is also rapidly bound to other soil components. Therefore, more study is needed to assess the role mobilized Ni from soil may play in elemental allelopathy.

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